Intrauterine corticosteroids for lung maturation: Observations of HPA axis function and cardiac autonomic balance in the neonate

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6 Intrauterine corticosteroids for lung maturation: Observations of HPA axis function and cardiac autonomic balance in the neonate

Leonhard Schäffer, Tilo Burkhardt, Maren Tomaske, Manfred Rauh, and Ernst Beinder

The putative long-term effects of antenatal glucocorticoid treatment for accelerating lung maturation in pregnancies at risk for preterm birth is still a matter of debate. Different animal models have shown that antenatal glucocorticoid exposure has a persistent impact on the hypothalamus-pituitary-adrenal (HPA) – axis regulation and that the developing cardiac autonomic system is sensitive toward antenatal glucocorticoids. In the human, the situation is less clear. Long-term observations in adolescents and young adults have rather focused on classic signs of disease manifestation such as blood pressure. Risk-factors for cardiovascular and metabolic disease, however, may transform into apparent clinical manifestation at a more advanced age when stability of these systems can be less compensated by the organism. Our studies analyzed whether a single course of antenatal betamethasone treatment has a direct and continuous effect on the developing HPA axis and the cardiac autonomic system. To this end, resting- and stress-induced salivary levels for cortisol, cortisone, and α-amylase as well as heart rate variability parameters as early signs of system alteration were analyzed. These studies were conducted in healthy newborns born late preterm or at term. Our studies show that a single course of antenatal betamethasone treatment induces a significant suppression of HPA-axis reactivity in healthy infants that is present several weeks later in early postnatal life. In contrast, the cardiac autonomic balance appears not to be primarily affected. Antenatal betamethasone treatment may therefore alter the HPA axis in a manner that predisposes these individuals toward a development of diseases in later life.

6.1 Introduction

Antenatal glucocorticoid administration is an established procedure in pregnancies with an imminent risk for preterm delivery. Before 34 weeks of gestation, maternal administration of synthetic glucocorticoids, such as betamethasone or dexamethasone, significantly reduces perinatal mortality and morbidity by maturational effects on the immature fetal lungs and decreases the risk for necrotizing enterocolitis and severe cerebral hemorrhage (Roberts and Dalziel, 2006).

Evidence from animal models, however, suggests that excess glucocorticoid exposure during intrauterine development may entail long-term alterations in the functionality and balance of homeostasis regulating systems. Indeed, major physiologic systems involved in the development of hypertension and the metabolic syndrome are glucocorticoid

sensitive targets during intrauterine development (Drake, Tang, and Nyirenda, 2007; Seckl, 2004). In the rat, maternal antenatal dexamethasone treatment results in elevated blood pressure in the offspring (Benediktsson et al., 1993; Woods and Weeks, 2005). Likewise, the maternal administration of dexamethasone in the sheep induces hypertension during adulthood (Dodic et al., 1998, 2002). Glucocorticoids are likewise involved in programming of glucose tolerance and metabolism. Again, in the rat as well as in the sheep, persistent alterations in glucose and insulin levels have been found in adult offspring after intrauterine glucocorticoid exposure (Moss et al., 2001; Nyirenda et al., 1998).

6.2 Vulnerability of the hypothalamic-pituitary-adrenal (HPA) axis

The hypothalamic-pituitary-adrenal (HPA) axis represents the organism's central organ of stress regulation and is involved in preservation of cardiovascular and metabolic stability, and elevated cortisol levels have been linked to arteriosclerosis and diabetes (Sapolsky, Romero, and Munck, 2000). The HPA axis and its key limbic regulator, the hippocampus, are especially sensitive to glucocorticoids because this system is tightly regulated through a feedback mechanism involving glucocorticoid (GR) and mineralocorticoid receptors (MR) at the level of the hippocampus, hypothalamus, and pituitary gland to inhibit HPA activity (Jacobson and Sapolsky, 1991). The HPA axis has been shown to be vulnerable to excess glucocorticoid exposure during its maturational stage (Kapoor, Petropoulos, and Matthews, 2008; Owen, Andrews, and Matthews, 2005). As such, overexposure to glucocorticoids results in attenuated hippocampal GR and MR expression. The resulting permanently increased cortisol levels and hypertension in the adult rats suggest an impaired negative feedback sensitivity of the HPA axis (Levitt et al., 1996). Further alterations at different levels of the HPA axis are likewise targets of glucocorticoid receptor alterations (Dean et al., 2001). Thus, glucocorticoid induced permanent alterations of these receptors may induce a resetting of the balance and reactivity of the HPA axis in later life.

6.3 Vulnerability of the sympathetic nervous system

The sympathetic nervous system (SNS) development has been shown to be sensitive toward antenatal glucocorticoids as well. In fact, the SNS is closely interconnected with the HPA axis as glucocorticoids provide direct signals for noradrenergic system maturation in the brainstem and thus on central noradrenergic activity (Slotkin et al., 1992). Furthermore, cardiac noradrenergic innervation is directly affected by glucocorticoids (Bian, Seidler, and Slotkin, 1993). Adrenergic cardiovascular drive and noradrenergic influences play a key role in the development or progression of hypertension and the metabolic syndrome in the adult (Grassi et al., 2009), making this system another key candidate for glucocorticoid induced long-term alterations in system balance. This notion is supported by experiments showing a sustained elevation of blood pressure and altered baroreceptor heart rate response in fetal sheep and the baboon and increased central and peripheral vascular resistance after glucocorticoid administration (Derks et al., 1997; Fletcher et al., 2002; Koenen et al., 2002; Schwab et al., 2000).

6.4 Findings in humans

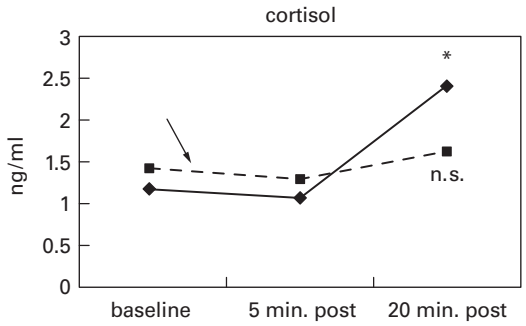
Findings of putative alterations of HPA-axis activity and SNS balance in the human after intrauterine glucocorticoid exposure are less clear. Premature neonates failed to increase cortisol levels in response to a stressor after intrauterine exposure to glucocorticoids for lung maturation (Davis et al., 2004, 2006). In the neonate as well as in a cohort of preterm born children at the age of 14 years, blood pressure was significantly increased after prenatal glucocorticoid treatment (Doyle et al., 2000; Kari et al., 1994). In contrast, a large prospective follow-up study did not find significant alterations in blood pressure and resting cortisol levels in 30-year-old adults after a single course of antenatal betamethasone treatment for lung maturation. Physiological functionality when challenging these systems was not available. Nevertheless, during a glucose challenge, clear indicators for the presence of insulin resistance were present in these individuals (Dalziel et al., 2005). In fact, studies have mainly focused on classical signs of disease manifestation; however, these signs may not become apparent until a later age when compensatory mechanisms fail after a certain threshold is reached.

If indeed the HPA axis and the SNS experience permanent alterations after intrauterine glucocorticoid exposure, these alterations should be detectible after birth. Considering the fact that these alterations may be mild and clinically not apparent during early life due to a high compensatory reserve being only "exhausted" over many years, we aimed to not only analyze HPA and SNS balance under resting conditions but also to challenge these systems.

A further rationale to analyze these systems in neonates was that in a normal postnatal period, temporarily challenged systems normalize in response to a normal postnatal environment, while permanently altered systems may become apparent, but postnatal compensatory system reactions may not have had the time to develop.

6.5 HPA axis functionality in neonates after intrauterine betamethasone treatment

To analyze the functionality of the HPA axis, we applied a simple noninvasive method by measuring cortisol and cortisone levels in salivary samples. Salivary cortisol reflects the unbound, active fraction of cortisol and is highly correlated with plasma cortisol levels (Calixto et al., 2002; Gunnar, 1989), making this method especially valuable for infants where blood sampling is less desired by parents. Likewise, to analyze for functionality under challenge, we made use of the routinely conducted heel-prick test 72–96 h after delivery, which has been shown to be an adequate stimulus for HPA-axis activation (Gunnar, 1992). Resting cortisol levels and cortisol levels after this challenge were measured. While resting levels of cortisol were comparable between the infants with antenatal betamethasone treatment and controls, we found that the neonatal HPA axis was significantly suppressed in infants with betamethasone treatment after the stimulus as these infants failed to adequately increase cortisol release (Schäffer et al., 2009; ▶Fig. 6.1). Because these infants were born near or at term and the mean interval between glucocorticoid treatment and delivery was more than 8 weeks, it is rather unlikely that these effects may only be a transient short-term effect considering that short-term effects of antenatal glucocorticoid exposure have been described to last for about 1 week after administration (Ballard et al., 1980; Parker et al., 1996). The



AuQ2 **Fig. 6.1:** Median cortisol levels during resting- (baseline) and post-stress induction (5 min. post, 20 min. post) phases in betamethasone exposed neonates (- - -) and controls (----). Arrow indicates stress induction. *$p < 0.05$, n.s.: not significant.

possibility that the decreased cortisol response in the study group would be the result of an increased conversion of cortisol to cortisone by an altered activity of the 11-beta hydroxysteroid dehydrogenase could also be excluded because cortisol and cortisone levels were strongly correlated in the study group, thus excluding this possibility. Therefore, it appears that a single course of antenatal betamethasone treatment for imminent preterm delivery has a suppressive effect on HPA-axis reactivity that persists at least for more than a mean of 8 weeks when these pregnancies continue and infants are delivered near or at term.

6.6 The cardiac autonomic system after intrauterine betamethasone treatment

A sensitive parameter to analyze early alterations in the cardiac sympathetic – parasympathetic balance is heart rate variability (HRV), which is a well-established noninvasive measure of cardiac autonomic control that has been shown to be related to hypertension (Guzzetti et al., 1991; Langewitz, Ruddel, and Schachinger, 1994) and to predict future adverse cardiovascular events in adults (Algra et al., 1993). Analyses of HRV have been suggested for putative prognostic use in children as well (Stewart, 2000). Analysis from 24 h Holter electrocardiogram (ECG) tracings calculating various parameters for short- and long-term analyses during active and resting states did not reveal any significant difference between betamethasone exposed infants and controls (Schäffer et al., 2010).

To further support our conclusion, we supplemented our electrophysiological studies by measuring salivary α-amylase levels during resting conditions and after a stressful stimulus (heel-prick test). Salivary α-amylase is secreted by acinar cells in the salivary glands that are richly innervated by both sympathetic and parasympathetic nerve fibers, influencing the release of α-amylase by classic neurotransmitters (Turner and Sugiya, 2002). Studies in humans and animals have suggested that the activation of the autonomic nervous system leads to a high activity of salivary α-amylase (Asking and Gjorstrup, 1987; Chatterton et al., 1996; Schneyer and Hall, 1991; Steerenberg et al., 1997). Furthermore, α-amylase levels have been found to be associated with cardiovascular

physiology and are suggested to be a surrogate for cardiovascular autonomic system balance (Granger et al., 2007), thereby making this parameter a promising indicator for cardiac autonomic function.

Interestingly, α-amylase levels rather decreased in betamethasone-exposed neonates in response to the stress event as compared to controls (Schäffer et al., 2010). We speculate that the slightly attenuated α-amylase response might be explained by the altered HPA-system sensitivity rather than by direct effects of steroid exposure on the sympathetic system as these systems are closely interconnected. Thus, it appears that the neonatal cardiac autonomic system is primarily unaffected by a single course of betamethasone treatment for lung maturation.

6.7 Summary

To our best knowledge, the presented studies are the first to analyze stress physiology in healthy term or near term delivered infants after a single course of betamethasone administration at a much earlier time in pregnancy. These early signs for alterations in the balance and activity of the HPA axis and the cardiac autonomic system may further contribute to the understanding of origins of disease development.

According to our results, a single course of antenatal betamethasone treatment for imminent preterm delivery entails alterations in HPA-axis responsiveness that persist into postnatal life. If this alteration of the HPA-axis balance really persists during life, it may represent a significant risk factor for the development of diseases in later life. In contrast, according to our results, cardiac autonomic balance appears to be preserved after exogenous glucocorticoid treatment at least in the neonate. However, due to the mutual interaction between the HPA axis and the SNS, this system may be subject to later alterations as the organism further matures.

Considering the beneficial effects of antenatal glucocorticoids to significantly reduce infant morbidity and mortality in pregnancies at risk for preterm delivery before 34 weeks of gestation, it is clear that these measures outweigh putative long-term risks according to current knowledge. Nevertheless, thorough evaluation in these cases is mandatory to ensure a clear indication for antenatal glucocorticoids. Prophylactic treatment in low-risk situations for preterm delivery should be avoided.

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